Hepatitis C Virus Load and Survival Among Injection Drug Users in the United States

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Persons chronically infected with hepatitis C virus (HCV), some of whom may be coinfected with HIV and human T-lymphotropic virus type II (HTLV-II), are at high risk for end-stage liver disease (ESLD). We evaluated whether ESLD death was associated with premorbid HCV RNA level or specific HCV protein antibodies among persons with or without HIV/HTLV-II coinfection in a cohort of 6,570 injection drug users who enrolled in 9 US cities between 1987 and 1991. We compared 84 ESLD descendents and 305 randomly selected cohort participants with detectable HCV RNA, stratified by sex, race, HIV, and HTLV-II strata. Relative hazard (RH) of ESLD death was derived from the proportional hazard model. Risk of ESLD death was unrelated to the intensity of antibodies against the HCV c-22(p), c-33(p), c-100(p), and NS5 proteins, individually or combined, but it increased with HCV RNA level (RH_{adj} = 2.26 per $\log_{10}$ IU/mL, 95% CI: 1.45-5.92). The association between HCV RNA level and ESLD death remained significant after adjustment for alcohol consumption (RH_{adj} = 2.57 per $\log_{10}$ IU/mL, 95% CI: 1.50-8.10). Deaths from AIDS (n = 45) and other causes (n = 43) were unrelated to HCV RNA (RH_{adj} = 1.14 and 1.29 per $\log_{10}$ IU/mL, respectively). HIV infection was not associated with ESLD risk in multivariate analyses adjusted for HCV RNA. Men had an increased risk of ESLD death in unadjusted analyses (RH = 1.92, 95% CI: 1.15-3.56) but not in multivariate analysis (RH_{adj} = 0.98, 95% CI: 0.48-2.88). Non-black patients were at increased risk for ESLD death (RH_{adj} = 2.76, 95% CI: 1.49-10.09). In conclusion, HCV RNA level is a predictor of ESLD death among persons with chronic HCV infection. (HEPATOLOGY 2005;42:1446-1452.)

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Persons with chronic hepatitis C virus (HCV) infection are at increased risk for cirrhosis and hepatocellular carcinoma.[1] The infection is common (60%-90%) among persons with a history of parenteral exposure, especially those with hemophilia or injection drug use, many of whom are coinfected with HIV.[1,2] A number of studies have shown that HCV RNA level (HCV load) is elevated among persons with HIV infection.[2-4] End-stage liver disease (ESLD) is related to persistent HCV infection (*i.e.*, detectable viremia),[5,6] but the relationship between ESLD and HCV RNA level is uncertain.[5-7] Compared with people infected only with HCV, those with HCV/HIV coinfection have an approximately 2- to 6-fold increased risk of ESLD,[8-12] leading to the hypothesis that HCV load may predict the risk of HCV-associated ESLD with or without HIV coinfection.

In a study of men with HCV/HIV coinfection and hemophilia, a higher prevalence of high anti-c100(p) and a lower prevalence of anti-c22(p) HCV antibodies each were associated with an increased risk of ESLD.[7] Altered HCV antibody patterns with HIV coinfection had been previously noted but had not been associated with ESLD,[13,14] and the association between antibody patterns and ESLD risk has not been replicated in another population.

Not only HCV and HIV, but also human T-lymphotropic virus type II (HTLV-II) infection is prevalent among injection drug users.[15] We have previously shown

Abbreviations: HCV, hepatitis C virus; HTLV-II, human T-lymphotropic virus type II; ESLD, end-stage liver disease; RH, relative hazard; EIA, enzyme immunoassay.

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Received January 27, 2005; accepted August 30, 2005.

Supported in part by the Intramural Research Program of the National Cancer Institute, NIH.

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Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.20938

Potential conflict of interest: Nothing to report.

that HCV load is significantly higher among persons coinfected with HTLV-II and HCV,[16] but the effect of HTLV-II coinfection on risk of ESLD among people infected with HCV is unknown. In this study, we estimated the risk of liver disease mortality by HCV RNA level and antibody patterns in a well-characterized population of US injection drug users, many of whom were infected with HCV, HIV, and HTLV-II.

Patients and Methods

Study Population

The subjects of our analysis were participants in the National Institute on Drug Abuse study to investigate risk factors and trends in HIV seroprevalence among injection drug users from methadone maintenance and detoxification clinics in 9 US cities.[15-17] The study enrolled 8,887 participants aged 18 years and over who were admitted to treatment between March 1987 and December 1991. Demographic characteristics and prevalence of HIV and HTLV-II infection in this population have been previously described.[15,17] Blood sample components, collected at the time of study enrollment, were stored at −70°C in a central repository until use. Informed consent was obtained from all study participants. The study protocol followed the human experimentation guidelines of the US Department of Health and Human Services, and the study was conducted with the approval of institutional review boards of the National Cancer Institute and the National Institute on Drug Abuse.

Study Subjects

Case Detection and Definition. Our analysis is a case-cohort design (Fig. 1). Vital status of the National Institute on Drug Abuse cohort participants through the end of 1998 was verified by linkage to the National Death Index as previously described.[18] After Asian/Pacific Islanders and Native Americans, who were too few for analysis, were excluded, 7,542 subjects with known HIV/HTLV-II infection status plus residual frozen sera were available for matching to the National Death Index for further evaluation. Excluding HTLV-I–positive and HTLV-I and -II co-positive subjects, a total of 6,539 subjects were matched, from whom 99 ESLD cases, *i.e.*, those who died with an underlying cause of liver disease (ICD-9 codes 570-576 and 070), were identified.[18]

Subcohort Selection. The 6,539 participants with known HIV/HTLV-II status were classified into 16 groups according to HIV/HTLV-II infection status (+/+, +/−, −/+, −/−), sex (M vs. F), and race (black vs. white and Hispanic combined).[15] We sought 25 participants in each of these 16 sex-, race-, and virus-specific strata. Selection was random whenever more than 25 subjects in a category were available. There were 12 and 23 eligible subjects in the $HIV^+/HTLV\text{-}II^+$ non-black female and male groups, respectively, all of whom were included. Of the 400 sought, we found 385 subjects (96.3%). Four of these 385 selected participants had died from ESLD. One person whose age at sample collection was ambiguous was excluded (Fig. 1).

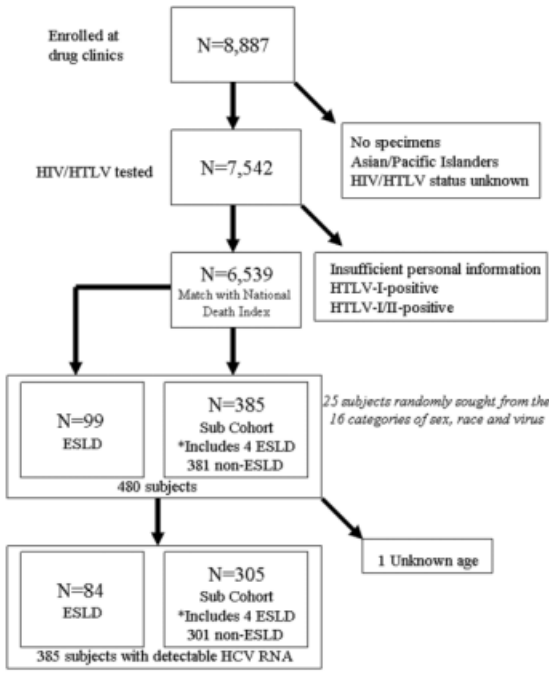

Fig. 1. Subject selection in the National Institute on Drug Abuse study.

Laboratory Methods

Sera were screened for antibodies to HIV using a whole-virus enzyme immunoassay (EIA) (DuPont NEN, Wilmington, DE), with a confirmatory Western blot (DuPont NEN). Detection of bands for gp120/160 and to p24 or gp41 defined HIV positivity. Sera also were screened for HTLV-I/II antibodies by recombinant p21e EIA (Cambridge Biotech, Worcester, MA) or whole-virus EIA (DuPont NEN or Genetic Systems, Redmond WA). Reactive sera in either EIA were confirmed by a Western blot (Biotech Research Laboratories, Rockville, MD). Reactivity to both p24 and rp21e was defined as HTLV-I/II–positive. Infection with HTLV-II was confirmed by synthetic peptide EIA (United Biomedical/Olympus, Hauppage, NY), recombinant protein-enhanced Western blot (Diagnostic Biotechnology, Singapore), or an algorithm comparing Western blot p24 and p19 band strength.[15,18,19] HCV antibody was detected by third-generation recombinant immunoblot assay (RIBA3.0, Chiron Corp., Emeryville, CA). HCV seropositivity was defined as the presence of at least two of the four bands

(c-100(p), c-22(p), c-33(p), or NS5) with an intensity of >1 on the 0-10 gray scale.[7] Serum HCV virus load was measured by the branched-DNA assay (HCV Quantiplex 3.0, Ortho Corp., Tarrytown, NY). Virus loads were converted to international units (IU)/mL, using the lot-specific conversion factor per the manufacturer's specification.

Statistical Analysis

Of a total of 479 participants (99 ESLD, 380 non-ESLD) who were eligible for analysis, 385 (84 ESLD, 301 non-ESLD) with detectable HCV RNA were included in the analysis. Duration of drug use, age at study enrollment, and HCV RNA level were categorized into quartiles. HIV/HTLV-II infection status was categorized into four groups (+/+, +/−, −/+, −/−). Intensity of each HCV antibody (c-100(p), c-22(p), c-33(p), and NS5) was dichotomous at its median value, consistent with our previous analysis.[7] Additional analyses were pursued, with whites separated *a posteriori* from Hispanics.

Survival methods were used for data analysis. The follow-up of the cohort participants extended from the date of study enrollment (phlebotomy) to the date of death or the completion of the death registry (default censoring date, December 31, 1998), whichever came first. Smoothed, cause-specific hazard of ESLD mortality was estimated stratified by sex, race, duration of injection drug use (enrollment age minus age at first injection), age at study enrollment, HIV/HTLV-II status and HCV RNA level. Univariate (Table 1) and multivariate (Table 2) Cox proportional hazard models were used to derive relative hazard (RH) for ESLD death with sex, race, duration of injection drug use, age at study enrollment, HIV/HTLV-II status, HCV antibody patterns, and HCV RNA level in quartiles and a continuous $\log_{10}$-transformed scale. In all analyses, we accounted for stratified case-cohort sampling design for our subcohort by an inverse of sampling weighted method.[16,20] In this approach, each study subject is weighted by the inverse of his or her probability of being sampled from the full cohort. In particular, all cases from the full cohort were selected in our study and were weighted by 1. In contrast, control subjects were weighted by the inverse of the sampling fraction of their 16 sex-, race-, and virus-specific strata. The sampling fraction for each stratum was obtained as the ratio of the total number of controls selected for analysis and the total number of controls available in the full cohort in the respective stratum. This weighting method allows each stratum to be represented proportional to its contribution in the population so that inference from the stratified sample can be generalized to the underlying population under study. Variance of parameter estimates were obtained by an appropriate bootstrap sampling method[21-23] that accounts for sampling variability both in the full cohort and in the selection of the subcohort. All statistical significance was based on two-sided tests at alpha level of 0.05.

Table 1. Characteristics of 385 Injection Drug Users With Detectable HCV RNA Levels in the National Institute on Drug Abuse Study, by End-Stage Liver Disease (ESLD) Status

	No. Subjects (%)			
	ESLD Descendents N = 84	Subcohort† N = 305	RH	95% CI
Sex			1.92	1.15–3.56
Male	68 (81.0)	162 (53.1)		
Female	16 (19.0)	143 (46.9)		
Race			1.61	1.02–2.85
Non-black	61 (72.6)	148 (48.5)		
Black	23 (27.4)	157 (51.5)		
Duration of drug use (yrs)				
≥23	26 (31.0)	68 (22.3)	3.11	1.38–8.80
18 - <23	27 (32.1)	74 (24.3)	1.75	0.84–4.41
11 - <18	18 (21.4)	93 (30.5)	1.12	0.43–3.22
<11	14 (15.5)	70 (22.9)		
Age at study enrollment (yrs)				
≥42	29 (34.5)	66 (21.6)	5.19	2.03–15.87
37 - <42	27 (32.2)	89 (29.2)	3.47	1.56–9.12
33 - <37	19 (22.6)	70 (23.0)	2.03	0.82–5.46
<33	9 (10.7)	80 (26.2)		
HIV/HTLV-II				
+/+	3 (3.6)	70 (23.0)	1.55	0.42–3.98
+/−	10 (11.9)	85 (27.9)	1.58	0.64–2.66
−/+	18 (21.4)	77 (25.2)	1.68	0.90–2.85
−/−	53 (63.1)	73 (23.9)		
HCV RNA quartile ($\log_{10}$, IU/mL)				
≥6.22	25 (29.8)	73 (23.9)	4.99	2.06–13.41
5.75 - <6.22	24 (28.6)	73 (23.9)	4.41	1.91–9.99
5.12 - <5.75	23 (27.4)	75 (24.6)	3.03	1.30–7.63
<5.12	12 (14.1)	84 (27.5)		
HCV antibody pattern				
C22(p) ≥ 9	33 (39.3)	108 (35.4)	1.27	0.75–2.36
C22(p) <9	51 (60.7)	197 (64.6)		
C100(p) ≥ 8	69 (82.1)	232 (76.1)	0.90	0.47–1.93
C100(p) < 8	15 (17.9)	73 (23.9)		
C33(p) ≥ 9	40 (47.6)	118 (38.7)	1.43	0.85–2.49
C33(p) <9	44 (52.4)	187 (61.3)		
NS5 ≥ 6	62 (73.8)	215 (70.5)	0.91	0.49–1.71
NS5 < 6	22 (26.2)	90 (29.5)		

Abbreviation: RH, relative hazard.

*95% CIs are from univariate Cox analyses. The referent groups are female, black, <11 years at first drug use, <33 years at study enrollment, HIV$^-$/HTLV-II$^-$, HCV RNA < 5.12 $\log_{10}$ IU/mL, c22(p) < 9, c100(p) < 8, c33(p) < 9, and NS5 < 6.

†Subcohort includes 4 randomly selected individuals who died from ESLD.

Results

Of 479 eligible participants, 469 (97.9%) had at least two of the four HCV antibodies by RIBA, of whom 94 (19.6%) did not have detectable HCV RNA. HCV RNA detection itself was not associated with age, sex, race, du-

Table 2. The Risk of Mortality From End-Stage Liver Disease (ESLD) Among 385 Injection Drug Users With Detectable HCV RNA Levels in the National Institute on Drug Abuse Study

Variable	Adjusted RH* (95% CI)
Sex	
Male	0.98 (0.48-2.88)
Female	1.00
Race	
Non-black	2.76 (1.49-10.09)
Black	1.00
Duration of drug use (yrs)	
≥23	2.66 (0.58-18.69)
18 - <23	1.40 (0.39-8.74)
11 - <18	1.21 (0.30-6.73)
<11	1.00
Age at study enrollment (yrs)	
≥42	5.04 (1.47-69.89)
37 - <42	3.48 (0.91-29.50)
33 - <37	2.31 (0.62-11.59)
<33	1.00
HIV/HTLV-II	
+/+	0.81 (0.09-2.19)
+/−	1.46 (0.28-2.68)
−/+	0.79 (0.14-1.46)
−/−	1.00
HCV RNA quartile (log_{10}, IU/mL)	
≥6.22	7.01 (2.35-58.58)
5.75 - <6.22	7.26 (2.32-66.34)
5.12 - <5.75	5.32 (1.76-34.33)
<5.12	1.00

Abbreviation: RH, relative hazard.
*Multivariate model includes all variables shown.

ration of infection, or HCV antibody pattern (data not presented). Characteristics of the 385 study participants with detectable HCV RNA by ESLD outcome are presented in Table 1. Their mean age was 38.2 years (range, 21.3-73.9 years) at study enrollment, and they were followed for a mean of 8.0 years (range, 0.1-12.5 years) until death or until December 31, 1998, whichever came first. The overall mean HCV load among the 385 participants with detectable HCV RNA was 5.58 log_{10} IU/mL.

As shown in Table 1, in the overall cohort men were significantly more likely than women to die with ESLD. Univariate Cox regression analysis indicated that men were at two-fold higher risk than women (RH = 1.92; 95% CI: 1.15-3.56). Non-black participants were significantly more likely to die with ESLD than were black participants (RH = 1.61; 95% CI: 1.02-2.85). Similarly, ESLD risk was increased among those with longer duration of drug use ($P = .001$) and among older participants compared with younger participants ($P = .003$). ESLD death was not significantly associated with HIV or HTLV-II infection (RH = 1.58; 95% CI: 0.64-2.66; RH = 1.68; 95% CI: 0.90-2.85, respectively, compared with neither infection). Likewise, HCV antibody patterns were not associated with ESLD mortality. ESLD mortality was associated with higher HCV RNA. The highest quartile of HCV RNA was associated with a 5-fold increase in risk of ESLD death compared with the lowest quartile (RH = 4.99; 95% CI: 2.06-13.41). A log_{10} increase in HCV RNA elevated the risk of ESLD death by 2.01-fold (95% CI: 1.35-3.19). Among HIV-negative participants, the risk of ESLD death was strongly related to HCV RNA level ($HR_{adj} = 2.57$ per log_{10} HCV RNA IU/mL; 95% CI: 1.50-8.10). The sampling adjusted mean HCV RNA level was 5.77 log_{10} IU/mL among those who died with ESLD compared with 5.34 log_{10} IU/mL among those who did not ($P = .001$).

As shown in Table 2, in a multivariate Cox model adjusting for sex, race, duration of drug use, age at study enrollment and HIV/HTLV-II status, HCV load remained a significant predictor of ESLD mortality. The highest quartile of HCV RNA was associated with a 7-fold increase in risk of ESLD death compared with the lowest quartile ($RH_{adj} = 7.01$; 95% CI: 2.35-58.58). A log_{10} increase in HCV RNA elevated the risk of ESLD death by 2.26-fold (95% CI: 1.45-5.92). Duration of drug use ($P = .0009$) and age at enrollment ($P = .0002$) were also associated with an increased risk. The association of ESLD with male sex, however, disappeared in the multivariate model (RH = 0.98; 95% CI: 0.48-2.88). The risk of ESLD mortality was higher among non-blacks compared with blacks ($RH_{adj} = 2.76$; 95% CI: 1.49-10.09). The risk among white Hispanics did not differ significantly compared with non-Hispanic whites ($RH_{adj} = 1.78$; 95% CI: 0.51-7.87), but it was higher compared with non-Hispanic blacks ($RH_{adj} = 3.63$; 95% CI: 1.63-16.39). The association of ESLD risk with HIV/HTLV status was further attenuated in the adjusted analysis, and none of the four HCV antibodies was associated with the ESLD risk. All 4 proteins could not be included in one model because of strong correlation between the levels of anti-c22(p) and anti-c33(p) ($R = 0.72$, $P < .0001$). Inclusion of anti-HCV proteins did not appreciably change the results in any model (data not presented).

Data on consumption of alcohol were collected by interview questionnaire. Because alcohol intake is a potential confounder, we examined the association of HCV RNA with ESLD risk in a model adjusting for level of alcohol consumption as a continuous variable. With this adjustment, a significant increase in risk of ESLD among subjects with higher HCV RNA was found ($RH_{adj} = 2.57$ per log_{10} HCV RNA IU/mL, 95% CI: 1.50-8.10).

The association between HCV load and risk of mortality due to AIDS and to other causes was examined separately in multivariate proportional hazard models. Neither AIDS-related death ($RR_{adj} = 1.14$) nor death

Table 3. Association of HCV RNA Levels With Cause-Specific Mortality in the NIDA Study

Cause of Death	Number of Cases	Adjusted RH per $\log_{10}$ HCV RNA*
ESLD	84	2.26 (1.45–5.92)
AIDS	45	1.14 (0.56–3.26)
Other	43	1.29 (0.78–2.71)

*Three separate models, each adjusted for sex, race, duration of drug use, age at study enrollment, and HIV/HTLV status.

related to other causes ($RR_{adj} = 1.29$) was associated with an increased HCV RNA level in this cohort (Table 3).

Discussion

We found herein that ESLD mortality was unrelated to HCV protein patterns, but was 2.26-fold higher per $\log_{10}$ IU/mL increase in HCV load. The association was specific for ESLD, as HCV load was not associated with death from AIDS or other causes.

In this cohort, we previously reported that drug users had excess risks of death from external and medical causes, including liver disease without HIV or HTLV-II infection. We observed that neither HIV nor HTLV-II infection was associated with increased liver disease mortality.[18] More recently, we found that HCV load was higher with either HIV or HTLV-II infection, but the difference (0.50 $\log_{10}$ IU/mL with HIV) observed in our previous study implies that HIV's effect on HCV load would increase hepatic mortality only 1.13-fold.[16] When controlled for HCV load, our present analysis again showed no independent association of HIV or HTLV-II infection with liver disease mortality.

This contrasts with other studies in which a 2- to 4-fold increased risk of ESLD death was found among persons infected with HIV.[2,24] Our study ascertained causes of death from death certificates, and we cannot exclude the possibility that ESLD deaths were ascribed to AIDS in HIV-infected subjects, a bias that would attenuate the relative risk of ESLD mortality associated with HIV infection. Widespread use of highly active antiretroviral therapy would have changed the mortality among HIV-infected individuals had we followed our study subjects longer. However, this is not an explanation for the lack of association of ESLD mortality with HIV infection in this study. Because we included no data after 1998, there was little opportunity for highly active antiretroviral therapy, which became available in 1996, to affect the outcome.[25] Moreover, the proportional hazard model we employed allowed us to evaluate the effect of risk factors on the cause-specific hazards, thus accounting for competing risks of death.

Our study suggests that in this cohort, HCV load plays an important role as a determinant of hepatic mortality with or without HIV or HTLV-II coinfection. Approximately 80% of persons with acute HCV infection progress to chronic hepatitis.[5] ESLD, the terminal stage of liver fibrosis and cirrhosis, develops in approximately 20% of persons chronically infected with HCV, resulting in death or liver transplantation.[5] Previous studies indicate that the rate of liver disease progression increases with age of over 40 years at HCV infection, male sex, high level of alcohol intake and coinfection with HIV or hepatitis B virus, but it has not been consistently associated with HCV genotype or load.[5,26-28] In fact, most studies have failed to find a direct association between HCV RNA level and ESLD. Among Irish women with HCV genotype 1b infection, higher serum HCV RNA levels were associated with severity of liver inflammation but not with the degree of fibrosis or serum alanine aminotransferase levels.[26] This is not surprising because HCV is not directly cytopathic and is thought to cause liver pathology through an inflammatory response. The lack of association between HCV RNA level and degree of fibrosis may be attributable to sampling error associated with biopsy or heterogeneity of the patient populations. Misclassification in the ascertainment of liver fibrosis would contribute to null results. Many of these negative results were based on cross-sectional studies, several of which had small numbers of heterogenous groups of patients. In contrast, we evaluated a relatively large number of US injection drug users who were followed for a mean of 8 years for ESLD mortality.

In a study of patients with hemophilia, HCV RNA level and alanine aminotransferase levels were highly correlated.[4] Two analyses of another hemophilia cohort found slightly but not significantly higher HCV loads among patients who progressed to ESLD compared with those who did not.[7,10] Of note, the latter analyses were confounded by HIV coinfection, and an association with HCV load may have been attenuated by inclusion of subjects with undetectable HCV RNA. Our analysis of injection drug users adjusted for HIV status and excluded participants with undetectable HCV RNA, eliminating these biases.

As there are approximately 3 million HCV-infected people in the United States,[29] the burden of disease associated with HCV is substantial. Coinfection with HCV and HIV, which is common among injection drug users and adults with hemophilia, makes the evaluation and care of individual patients, as well as public health interventions, especially challenging. HTLV-II infection does not appear to contribute to ESLD risk, despite its association with higher HCV load in our cohort and a syner-

gistic effect of HTLV-I, a closely related retrovirus, on hepatitis incidence and liver cancer death.[16,30]

In our study, HCV antibody patterns were unrelated to liver disease mortality, thus failing to support a reported association among patients with hemophilia.[7] There were, however, numerous differences between these two studies, including size and endpoint (84 deaths vs. 28 incident cases and controls), interval from phlebotomy to end point (mean 8 years before death vs. 2.6 years before incident ESLD), age at HCV infection (median 19 years vs. 8 years in drug users and hemophiliacs, respectively), and prevalence of HIV (50% of subcohort of drug users vs. 100% of hemophiliacs).[7,16] Further study of whether HCV antibody patterns are related to risk at various intervals prior to ESLD, particularly in the absence of HIV coinfection,[31] would be of interest.

Consistent with previous reports,[6] we found that risk of ESLD was lower for blacks than non-blacks. ESLD risk in our study also tended to be higher for Hispanic whites than for non-Hispanic whites. Numerous gene loci, such as HLA class II and cytokine genes have been investigated in relation to the outcome of HCV infection. DQB1*0301 has been associated with spontaneous clearance of HCV infection.[32,33] In another study, polymorphisms in the promoter region of the interleukin-10 gene, an anti-inflammatory cytokine, also were related to the development of HCV-related liver fibrosis.[34] In addition, recent studies showed that polymorphisms in the gene encoding monocyte chemotactic protein (MCP)-1, a potent chemokine,[35] and in the hemochromatosis gene (HFE)[36] were both associated with an increased predisposition to hepatic inflammation, fibrosis and cirrhosis among HCV-infected patients. We speculate that the observed racial difference in ESLD risk may be, in part, due to differences in host genetics, as the frequencies of most, if not all, of these alleles differ by race.

Excessive alcohol consumption is associated with acceleration of HCV-related liver disease progression, in part through an increase in HCV replication.[37] Thus, we performed additional analysis on 75% of the study subjects for whom alcohol data were available. The association of HCV RNA with ESLD death was observed after adjustment for alcohol intake (HR_{adj} = 2.57 per $\log_{10}$ HCV RNA IU/mL; 95% CI: 1.50-8.10), suggesting that confounding by alcohol intake does not explain the observed association.

Our study had several limitations. First, our HCV RNA levels and their association with liver mortality may have been confounded by CD4 count. We were unable to thoroughly examine this possibility due to lack of information on CD4 count. However, we found a significant association of HCV RNA with ESLD death in the subgroup of subjects who were free of HIV infection (HR = 2.76 per $\log_{10}$ increase in HCV RNA level), suggesting that the association is valid among persons with normal levels of CD4 lymphocytes.

Second, we lacked data to evaluate the contribution of chronic hepatitis B virus infection to ESLD risk. Compared with HCV infection alone, hepatitis B virus coinfection worsens hepatitis but also inhibits HCV replication and often reduces HCV load.[38,39] Thus, had we been able to exclude, stratify, or adjust for subjects with chronic hepatitis B virus infection, who are likely to have a high ESLD risk despite a relatively low HCV load, our observed association between liver mortality and HCV viral load would have been magnified.

Highly active antiretroviral therapy could confound the relationship between ESLD mortality and HCV RNA levels,[39] but our subjects were enrolled and plasma samples collected more than 5 years before highly active antiretroviral therapy was available.[25] Our subjects also were unlikely to have received interferon or another anti-HCV therapy prior to their enrollment in 1987-1991. In addition, our use of the proportional hazard model takes competing risks of death into account.

Finally, although the association of HCV RNA with AIDS and other causes of death was not significant, the specificity of the association should be interpreted with caution. With our case-cohort design focused on ESLD, fewer deaths due to AIDS and other causes were included. Moreover, as shown in Table 3, the 95% CI for the HR of all three causes of death overlapped.

In conclusion, we found that the risk of death from ESLD more than doubled with each $\log_{10}$ increase in HCV RNA. Corroboration in other populations is needed for what appears to be a previously underappreciated role of HCV RNA as a predictor of mortality from ESLD. If confirmed, one might argue for use of antiviral therapy to reduce HCV RNA level, even if the virus cannot be eliminated, to reduce mortality related to ESLD.

Acknowledgment: We thank Myhanh Dotrang for preparing data for analysis, and Eric Engels for the review of the manuscript.

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